

AMENDMENTS TO THE SPECIFICATION

Please replace the paragraph at page 3, lines 4 to 17, with the following:

Intravenous immunoglobulin preparation (e.g. IGIV[;]) Baxter and others is a highly purified preparation of IgG commercially available and is used in the treatment of patients who have No, or very low levels of antibody production. Immunoglobulin preparations include those available from the following manufacturers: Baxter (US) [[eg]]e.g. ~~Gammagard~~GAMMAGARD®, Isiven (Antimo Naples, Italy), Omrix (Tel-Hashomer, Israel), Miles (Biological Products Division, West Haven, CT), Scalvo (Lucca, Italy), Sandoz (Novartis, Basel, Switzerland [[eg]]e.g. ~~Sandoglobulin~~SANDOGLOBULIN®), Biotest Diagnostic Corporation (Deville, NJ). Examples of immunoglobulin preparations are ~~Gammagard~~GAMMAGARD S/D®, ~~Gammar~~GAMMAR IV®, ~~Gammar~~GAMMAR-P IV®, ~~Gammimune~~GAMMIMUNE N®, ~~Iveegam~~IVEEGAM®, ~~Panglobulin~~PANGLOBULIN®, ~~Polygam~~POLYGAM S/D®, ~~Sandoglobulin~~SANDOGLOBULIN®, ~~Venoglobulin~~VENOGLOBULIN®. Immunoglobulin preparations typically contain some IgM as well as IgG. Trace amounts of IgM are presentn ~~Gammagard~~GAMMAGARD®. Pentaglobin (Biotest) is an enriched IgM preparation which as been used for treatment of SARS.

Please replace the paragraph at page 8, lines 25 to 28, with the following:

Figure 1: Effect of pre-incubation of high antiphospholipid antibodies (aPLs) titer serum with human pooled immunoglobulin ~~Gammagard~~GAMMAGARD® on Annexin V binding to human umbilical endothelial cells (HUVECs): flow cytometry analysis after 24 hrs culture.

Please replace the paragraph at page 12, lines 20 to 30, with the following:

The cells were harvested non-enzymatically with Cell Dissociation Solution (CDS; Sigma-Aldrich, St. Louis, MO, USA). HUVECs were carefully pooled with supernatants, to exclude selective loss of detached floating EC, and centrifuged at 1200 rpm for 7 min. After resuspension in 100µl of Annexin V-binding buffere (Molecular Probes Inc, Eugene, OR, USA) samples were stained with 5mg/ml of Annexin V-FITC (Mol. Probes) and incubated for 15 min on ice. Shortly before acquisition 1mg/ml of propidium iodide (PI; R&DSYSTEMS Europe LTD, Abingdon, UK) was added. Analysis was performed on a FACScan flow cytometer (BD Biosciences, San Jose, CA, USA) equipped with CellQuest[™]CELLQUEST[™] cell sorting software. During acquisition a gate was set to exclude events smaller than 230 on linear FCS and SSC. For each sample 10000 events were collected.

Please replace the paragraphs at page 13, lines 1 to 32, with the following:

Immunostaining was performed on human plaques, characterized previously. Plaques were collected from 12 patients undergoing carotid endarterectomy after transient ischemic attacks. All specimens contained advanced atherosclerotic lesions. As a control macroscopically healthy mesenteric artery was obtained from unrelated bowel resection. The cryostat sections were fixed for 20 minutes in 2% paraformaldehyde in PBS (Sigma Chemicals) at 4°C and stored at -70°C. After blocking endogenous peroxidase, the sections were incubated overnight with monoclonal antiAnnexin V antibody (Alexis Biochemicals, Corp., Lausen, Switzerland) of mouse type IgG2a, anti-CD68 (DakoCytomation, Glostrup, Denmark) or anti-CD31 (Monosan, Uden, The Netherlands). Irrelevant mouse IgG2a (Serotec Ltd, Oxford, UK) served as negative control. All antibodies were diluted in 1%BSA-0.02%NaN₃ in PBS. After washing, 1% normal horse serum in PBS was used. Secondary antibody-biotinylated horse anti-mouse immunoglobulin (Vector Laboratories, Burlingame, CA, USA) was added. The ABC peroxidase Elite[™]ELITE[™] kit was used (Vector Laboratories). The staining was revealed with diaminobenzidine (Vector Laboratories) and counterstaining was done with

haematoxyline. All sections were analyzed on a Leica DMRXA microscope (Leica, Wetzlar, Germany).

Preparation of aPC

Total IgM or IgG fraction was separated from commercially available pooled human immunoglobulin (~~Gammagard~~GAMMAGARD®) at 50mg/ml using HiTrap IgM or IgG columns (Amersham Biosciences). Antibodies against phosphorylcholine (PC) were eluted after loading IgM or IgG fraction on NHS-Sepharose columns coupled to PC conjugated either to keyhole limpet haemocyanin protein (KLH)(1 or 5 mg/ml) or to bovine serum albumin (BSA) (1 mg/ml) followed by BSA-only column. PC-BSA (Phosphorylcholine-Bovine Serum Albumin) and PC-KLH was purchased from Biosearch Technologies, INC (Ca, USA). Eluted fractions were buffer-exchanged on PD-10 columns and concentrated with Millipore ~~Centricon~~CENTRICONE® devices. Procedures were performed according to instructions given by manufacturers. The concentration of IgM aPC prepared was typically 50 µg/ml, and the concentration of IgG aPC was typically 30µg/ml.